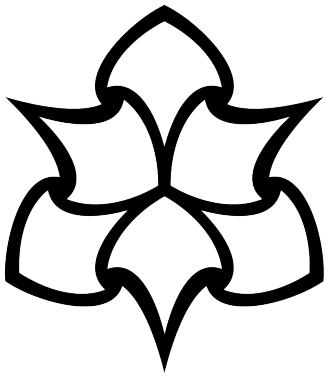

Clash of the Cascades: Release the Inhibition

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Skeletal muscle serves numerous functional and metabolic roles in human health. Consequently, understanding the mechanisms that regulate skeletal muscle hypertrophy is pertinent to improving the quality of life, particularly in ageing and diseased populations, but also for performance in athletic populations. Resistance exercise (RE) potently stimulates muscle protein synthesis (MPS), and thus chronic RE (i.e., resistance training) results in muscle hypertrophy. Mechanistically, RE activates the mammalian target of rapamycin complex 1 (mTORC1) signaling cascade and, in turn, regulates several key downstream components (e.g. Ribosomal protein S6 kinase 1 (p70S6K1)) that underpin increases in MPS. AMP-activated protein kinase (AMPK) is activated by cellular energy depletion, such as low muscle glycogen and increased AMP:ATP and ADP:ATP ratios, and is purported to inhibit mTORC1 activity.

Whether muscle glycogen content, independent or dependent of AMPK, influences mTORC1-S6K1 signaling and MPS remains unknown; so, a recent article in *The Journal of Physiology* by Knudsen et al. sought to address this aperture in research (Knudsen *et al.*, 2020). In a parallel fashion, wild type (WT) and dominant negative α_2 AMPK kinase dead (AMPK-KD) transgenic mice (12-24 weeks old) were each allocated to untreated, normal glycogen (CON) or high glycogen (GLYC) groups. For five days before testing, CON mice underwent standard feeding with oral gavage vehicle control and GLYC mice underwent "glycogen loading"; specifically, phosphorylase inhibitor CP 316819 and sucrose were administered to increase muscle glycogen content. On the test day, mice were fasted for two hours and anaesthetized before RE. Unilateral *in situ* contractions simulated RE and permitted the non-exercised leg to serve as a within-animal, rested control. RE consisted of quadriceps muscle stimulation for nine 1-minute contraction bouts (10 V, 0.1 ms at 100 Hz for 3 seconds every 10 seconds), each separated by 30 seconds of rest. Several markers of mTORC1 and AMPK activity were measured with immunoblotting immediately (0 h) and 4 hours (4 h) post-RE, including $AMPK^{S485/491}$, $S6K1^{T389}$, $rpS6^{S235/236}$ and $4E\text{-}BP1^{T36/45}$ (refer to full text for complete list). Four hours post-RE, MPS was measured using the SUnSET method, and muscle glycogen content was measured with a fluorometric method as glycosyl units after HCL hydrolysis.

The authors reported several exciting and novel findings. First, in untreated mice, RE-induced mTORC1-S6K1 signaling was not affected by AMPK. Understandably, $AMPK^{S485/491}$ activity was only significantly increased in WT mice immediately following RE; however, no between-genotype activity differences were observed for several downstream targets of mTORC1, including S6K1 and rpS6. Second, following RE, MPS was impaired in untreated AMPK-KD mice compared with untreated WT mice. Specifically, MPS increased significantly

above rested values in the WT (~40%) but not AMPK-KD mice (~15%). Third, the GLYC treatment, compared to the CON treatment, increased mTORC1-S6K1 signaling in WT but not AMPK-KD mice following RE; namely, S6K1 and rpS6 activation increased in only WT mice. Importantly, AMPK-KD mice exhibited lower (~25%) resting glycogen compared to WT mice. Glycogen content was significantly elevated above resting values in WT (50%) but not AMPK-KD (30%) mice, despite glycogen phosphorylase^{Ser15} being similarly supressed and unaffected by contraction in both genotypes. Lastly, within genotypes, GLYC appeared to further augment MPS compared to CON in AMPK-KD but not in WT mice. Moreover, when protein synthesis data from untreated and vehicle treated CON mice were combined within-genotypes, protein synthesis increased significantly less in AMPK-KD (25%) than WT mice (60%). Since GLYC appeared to increase MPS in AMPK-KD mice, the authors suggested that reduced glycogen content may diminish MPS independent of mTORC1 signaling. Overall, these data suggest that following RE: 1) mTORC1-S6K1 signaling can occur independently of AMPK, 2) a disconnect exists between mTORC1-S6K1 signaling and MPS, and 3) muscle glycogen content can influence mTORC1-S6K1 signaling and MPS via an unknown mechanism.

AMPK is frequently reported to blunt MPS via reduced mTORC1 activation in both mice and humans, and AMPK has subsequently been considered a negative regulator of mTORC1 (i.e., the concurrent training effect). Nevertheless, Knudsen et al. demonstrated that mTORC1 signaling occurs independent of AMPK. The lack of between-genotype differences in downstream targets of mTORC1 such as S6K1, rpS6 and 4E-BP1 activity – regardless of increased AMPK activity or absence – suggests that AMPK is not necessarily a negative regulator of mTORC1 following RE. While AMPK *can* inhibit mTORC1 activation, the present study indicates that increased AMPK activity can exist without inhibiting mTORC1 if sufficient

mTORC1-activating signals are present; thus, AMPK is not necessarily a tyrannical inhibitor of mTORC1. Rather, mTORC1-activating and -inhibiting signals appear to contend with each other for mTORC1 activity, with glycogen content influencing the outcome via an unknown mechanism. Previously, researchers have associated increased AMPK activity with decreased MPS during RE while also showing that both MPS and AMPK activity were significantly elevated 1 hour post-RE (Dreyer *et al.*, 2006). Similar to Knudsen et al. these researchers suggested that signals promoting MPS, such as from mTOR and S6K1, may override the inhibitory effects of AMPK on MPS (Dreyer *et al.*, 2006). Altogether, emerging evidence continues to suggest the inhibitory effect of AMPK on mTORC1 signaling and MPS can be abolished by mTORC1-activating signals.

The data from Knudsen et al. highlight a disconnect between mTORC1 signaling and MPS. For example, MPS was impaired in AMPK-KD mice when compared with WT mice, despite similar mTORC1 signaling. Researchers have shown that mTORC1-dependent signaling is necessary for mechanical load-induced (i.e., RE) hypertrophy and S6K1 phosphorylation, but that mTORC1-dependent signaling is not necessary for mechanical load-induced increases in MPS (You *et al.*, 2019). Specifically, the authors suggested that mTORC1-independent mechanisms may be responsible for the observed increases in MPS while mTORC1-dependent mechanisms are required for the post-translation modifications and autophagy necessary for hypertrophy (You *et al.*, 2019). Moreover, the mTORC1-dependent and -independent mechanisms governing MPS have been shown to differ temporally following RE and may each be necessary for recovery from RE (West *et al.*, 2016). While several potential mechanisms warrant discussion, explaining the malalignment in mTORC1 signaling and MPS reported by Knudsen et al. requires further investigation.

The apparent effect of muscle glycogen on mTORC1 signaling and MPS is an intriguing observation; particularly, the additive effect of glycogen content on mTORC1 signaling and MPS in WT and AMPK-KD mice, respectively. Glycogen content is infrequently considered when studying RE and MPS but rather considered during prolonged, non-hypertrophic exercise. Investigating the role of glycogen content in mTORC1 signaling and MPS may reveal an overlooked connection between these cellular processes and improve our understanding of the mechanisms that govern skeletal muscle mass.

When interpreting the results from Knudsen et al., an important limitation should be considered - *in situ* electrical stimulation, though a valuable technique, is not a direct RE-mimetic and this may preclude the translation of the results to humans. Despite increasing mTORC1-S6K1 signaling, contractions at a stimulation frequency of 100 Hz likely recruits all motor units and would not mimic the normal recruitment pattern – the size principle – typically observed during voluntary RE in humans (Ljubicic *et al.*, 2005). While the authors' observations may indeed represent the true nature of these cellular processes, alternate models of RE should be utilized to confirm these results.

Future research should endeavour to disentangle the interaction between mTORC1 signaling and MPS. Specifically, investigating the role of skeletal muscle glycogen content on mTORC1 signaling and MPS, while addressing the limitations discussed herein, is intriguing and could yield novel results. Given the multitude of intricate responsibilities served by mTORC1 and additional mechanisms underpinning MPS, incorporating the emerging "omic" techniques (e.g., metabolomics) could help pinpoint the central regulators of hypertrophy. Animal models certainly play a key role in scientific discovery, but researchers should work quickly to translate these findings to human models - since the ultimate objective is to improve skeletal muscle

health in various human populations. Building upon results from Knudsen et al., researchers would do well to investigate the influence of glycogen manipulation on mTORC1 signaling and MPS within various human populations. Specifically, the effects of increased muscle glycogen content (i.e., "glycogen loading") within an athletic population or the inability to breakdown glycogen in McArdle's disease patients would each provide an interesting avenue for future research.

As with any well-conducted study, upon completion, the findings generate more questions than answers. Knudsen et al. report an intriguing series of experiments that contribute nicely to the muscle physiology field and should be commended for their work. In addition to showing that AMPK is not necessarily an oppressive regulator of mTORC1, they highlight disparities between mTORC1 signaling and MPS while adding muscle glycogen to the multitude of possible mechanisms underpinning hypertrophy.

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Additional Information

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